Tolerance of three European native species of crayfish to hypoxia.

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Short Research Note

Tolerance of three European native species of crayfish to hypoxia

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Abstract

Species that can act as indicators of ecosystem health offer a valuable tool in the management of natural resources. Crayfish have been suggested as bioindicators of water quality in Europe and at least one species (*Austropotamobius pallipes*) has been studied to determine its tolerance to pollution and its potential as a bioindicator. The genus *Austropotamobius* includes three crayfish species native to western Europe: *A. pallipes*, *A. italicus* and *A. torrentium*. It was hypothesised that because of their geographical and habitat distribution, the three *Austropotamobius* species might vary in their value as a bioindicator of water quality. Crayfish of species *A. pallipes* and *A. italicus* were subjected to three different treatments: hypoxia (treatment 3, approx 3 mg l^{-1} O_2), light hypoxia (treatment 2, approx 5.5 mg l^{-1} O_2) and normoxia (treatment 1, control, approx 8.5 mg l^{-1} O_2). *A. torrentium* crayfish were only subjected to treatment 1 (control) and 3. Variations in haemolymph sodium, calcium and chloride were used as a biomarker and concentrations were measured before and after treatment to evaluate hypoxia-induced stress. Significant differences in the concentrations of sodium between the control groups (treatment 1, normoxia) and the experimental groups (treatment 3, 3 mg l^{-1} O_2) were found in the species *A. pallipes* and *A. torrentium*. Groups of *A. italicus* did not show any significant difference between treatments in sodium concentrations but in chloride concentrations. Crayfish of all three species demonstrated a disruption in the ion exchange process in hypoxia, but all tolerated very low oxygen concentration for an extended period of time.

Introduction

Species that can act as indicators of ecosystem health offer a valuable tool in the management of natural resources. Several species have been studied and suggested as biological indicators of water quality in lotic habitats. Scientists and managers in Europe have been interested in crayfish for many years, partly because of the drastic decline of native species populations (Vigneux & Souty-Grosset, 2000), but also because of their social and economic importance in many countries (Reynolds & Souty-Grosset, 2003). Crayfish have been suggested as bioindicators of water quality in Europe and at least one species (*Austropotamobius pallipes*) has been studied to determine its tolerance to pollution and its potential as a bioindicator (Gallagher, 2002; Demers & Reynolds, 2003; Lyons & Kelly-Quinn, 2003; Trouilhé et al., 2003).

The genus *Austropotamobius* includes three crayfish species native to Western Europe: *A. pallipes*, *A. italicus* and *A. torrentium*. *A. pallipes* and *A. italicus* have just recently been separated into two separate species (Santucci et al., 1997; Grandjean et al., 2002) and some authors still consider them as two subspecies (*A. pallipes pallipes* and *A. pallipes italicus*). *A. pallipes* is distributed in France, Ireland, Great Britain and north-west Italy while *A. italicus* is found in Spain, Italy, Austria and Dalmatia (Grandjean et al., 2002; Holdich, 2002). *A. torrentium* is mostly found around the alpine region and central Europe (Holdich, 2002).

Austropotamobius pallipes was thought to be sensitive to pollution (Jay & Holdich, 1981; Holdich & Reeve, 1991; Reynolds et al., 2002) but recent studies have shown that this species is quite tolerant to eutrophication (Troschel, 1997; Demers & Reynolds, 2002, 2003; Gallagher, 2002; Trouilhé et al., 2003). Less information is available on the other species. However, it was suggested that because of their different geographical distribution, the three *Austropotamobius* species might have a different value as a bioindicator of water quality. Although the three species are found in similar habitats such as upland streams with rocky substrate and shaded banks, each species is found in a distinct geographical area. This means that each species is exposed to different climatic conditions.

Hypoxia, temporary or permanent, is often a consequence of eutrophication and organic enrichment (for example Karim et al., 2002; Parr & Mason, 2004). *A. pallipes* is known to tolerate environmental hypoxia for prolonged periods of time (Demers, 2003), but little is known about the tolerance of *A. italicus* and *A. torrentium* to low oxygen concentrations.

Osmoregulation has been used as a biomarker in fish (Eddy, 1981; Wendemeyer & McLay, 1981) and has also been studied in crustaceans (Bjerregaard & Vislie, 1985; Fjeld et al., 1988; Boitel & Truchot, 1990; Ahern & Morris, 1998). Lignot et al., 2000, in their extensive literature review on osmoregulation as a biomarker in crustaceans, came to the conclusion that variations in osmotic and ionic regulation can be considered as a warning of sublethal stress, such as that caused by hypoxia. Osmoregulatory capacity (OC) is defined for a given species as the difference between the osmotic pressure of the hemolymph and of the external medium at a given salinity (Charmantier et al., 1989). Since the ions Na^+ and Cl^- make up 90% of the osmotic pressure in crustaceans (Prossner, 1973; Castille & Lawrence, 1981), ionic regulation has also been used as a biomarker (Caldwell, 1974; Fjeld et al., 1988; Jeberg & Jensen, 1994). The aim of this research was to test the different tolerance to hypoxia to the three native crayfish of Western Europe using haemolymph ionic concentrations as a biomarker.

Materials and methods

Austropotamobius pallipes crayfish were obtained by trapping in the Magot river, Deux-Sèvres Département, with the permission of the 'Conseil Supérieur de la Pêche'. Twenty-one crayfish were caught. Specimens of *Austropotamobius italicus* were hand caught in the Gattaia river, Mugello province, and brought to Poitiers by train. All 24 crayfish survived transportation. *Austropotamobius torrentium* crayfish were hand caught in Kammel river, Bavaria. They were brought back to Poitiers by car and only 12 crayfish survived transportation. All crayfish were intermolt. Table 1 presents some of the characteristics of the sites where crayfish were caught.

Although all rivers were small upland streams, the Italian site was in a forested area while the two other sites were in farmed areas. This is reflected (in case of the Magot) in the important dissolved oxygen variation and the high nitrate concentrations encountered. Despite the low oxygen concentrations measured, the crayfish population in the French stream is one of the most dense in the region. The Italian stream can be considered unaffected by human activity and the population at the site is healthy. The values for several variables in Table 1 were not available for the German stream, but biological quality was also rated as 'good'. Substrate was very similar at all sites.

Crayfish of the three species were held individually in 20 l aquaria all linked to a common filtration and cooling system and allowed to acclimatise for 5 days. Temperature was kept around 16 °C and the photoperiod imposed was the natural photoperiod relayed to the system *via* a receptor located outside. Crayfish of species *A. pallipes* and *A. italicus* were subjected to three different treatments: normoxia (treatment 1, control, approx 8.5 mg l^{-1} O_2, 85% saturation or 17.7 kPa), light hypoxia (treatment 2, approx 5.5 mg l^{-1} O_2, 55% saturation or 11.5 kPa) and

Table 1. Physical and chemical variables of the three sites where crayfish were caught. Temperatures as well as dissolved oxygen and nitrate concentrations are minimum and maximum recorded yearly

Variables	*A. pallipes*	*A. torrentium*	*A. italicus*
River	Magot (France)	Kammel (Germany)	Gattaia (Italy)
River width	2 m	6 m	3 m
Substrate	Rocky	Rocky	Rocky
Riparian vegetation	Yes	Yes	Yes
Land use	Farmland	Farmland	Forest
Temperature	5–20 °C	Na	5–17 °C
Dissolved O_2	5–11 mg l^{-1}	Na	7–12 mg l^{-1}
Nitrate	15–57 mg l^{-1}	Na	1–10 mg l^{-1}

hypoxia (treatment 3, approx 3 mg l^{-1} O_2, 30% saturation or 6.3 kPa). *A. torrentium* crayfish were only subjected to treatments 1 (control) and 3. For treatments 2 and 3, the desired oxygen concentration was maintained by bubbling nitrogen through the water. There were 8 crayfish per treatment for *italicus*, 7 for *pallipes*, 6 for *torrentium*; the difference of n for each species is due deaths of crayfish during transport.

Crayfish were acclimated to the aquaria for 5 days in normoxia before the first haemolymph sample was taken. They were then submitted to the experimental conditions for another 12 days and a second haemolymph sample was taken after this period. Crayfish were not fed during the acclimation period and for 5 days before the second haemolymph sample. At other times, they were fed with dry eel pellets.

Haemolymph samples of about 0.3 ml were taken at the base of the third walking leg with a 0.5×1.6 mm needle and 1 ml syringe. A small volume of anticoagulant (3.5 µl of 200 mM phenylthiocarbamide) was added and the samples were frozen at −80 °C for later analysis. Haemolymph samples were diluted by a factor of 1000 (50 µl in 50 ml; three replicate dilutions were done per sample). Sodium (Na) and calcium (Ca) concentrations were measured by plasma mass emission (ICP) using an Optima 4003 DV-Perkin Elmer. Chloride (Cl) was measured by ionic chromatography using a Vydac column (302 IC 4.6).

Measured concentrations were analysed by an analysis of covariance, using concentrations after (after 12 days treatment) as dependant variables and concentrations before (after 5 days acclimation) as covariables, with SPSS 12.

Results

Significant differences in the concentrations of sodium between the control groups (treatment 1, normoxia) and the experimental groups (treatment 3, 3 mg l^{-1} O_2) were found in the species *A. pallipes* and *A. torrentium* (Table 2 and Fig. 1). On average, *A. pallipes* crayfish in the control group (treatment 1) had a haemolymph sodium concentration 15% higher then crayfish kept in hypoxia (treatment 3). For *A. torrentium*, the difference between the control and the experimental groups was only 6% higher, but it is highly significant.

The three groups of *A. italicus* did not show any significant difference in sodium concentrations between treatments but in chloride concentrations

Table 2. Significant probability values and associated r-squared of the ANCOVAs of the ion concentrations for each species. Mean differences of ion concentrations between treatments are also shown. Only statistically significant pairs are included

Species	Ion	*r*-squared (ANCOVA)	Significance	Treatment	Mean difference mmol (g l^{-1})
pallipes	Na	0.653	p: 0.001	1–3	34.8 (0.8)
torrentium	Na	0.863	$p < 0.0005$	1–3	13.0 (0.3)
italicus	Cl	0.752	p: 0.001	1–3	−14.1 (−0.5)

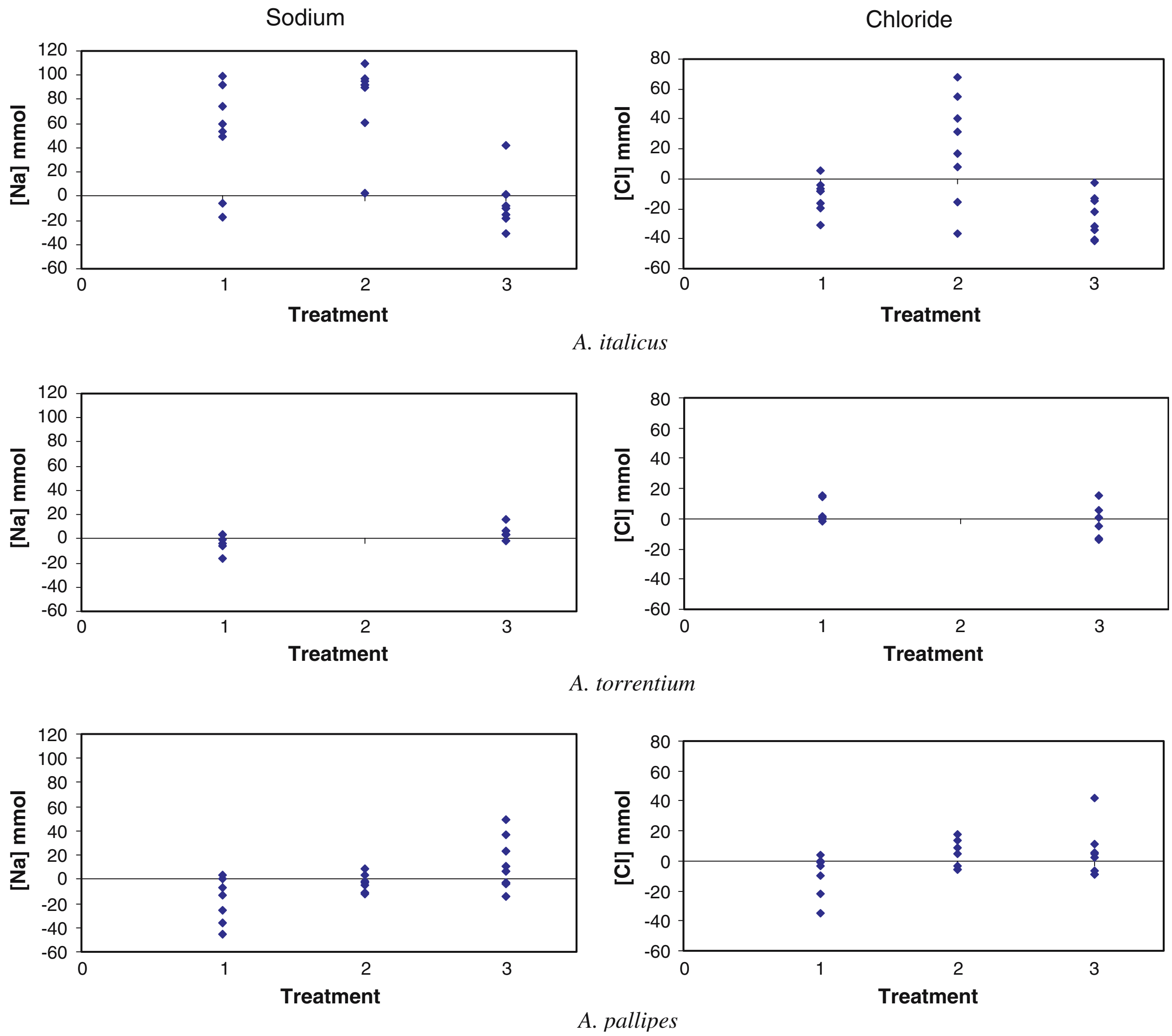

Figure 1. Difference in the sodium and chloride haemolymph concentrations between before treatment and after treatment. Treatment 1: control 85% O_2 saturation; treatment 2: light hypoxia 55% O_2 saturation; treatment 3: hypoxia 30% O_2 saturation.

(Fig. 1). However, individuals in treatment 2 showed great variability in chloride concentrations, contradicting the expectation of equal variance in the ANCOVA. When only treatments 1 and 3 are compared (Table 2), mean differences show that *A. italicus* crayfish kept in hypoxia (treatment 3) had a haemolymph chloride concentration 10% higher than those kept at normoxia (treatment 1). Chloride concentrations did not show consistent results in *A. pallipes* or *A. torrentium*.

Calcium concentrations in the haemolymph did not vary significantly between groups in any of the species.

Discussion

The most important symptom of eutrophication in terms of water chemistry is fluctuation in oxygen saturation. A high biological oxygen demand owing to organic pollution or increased vegetation growth will result in hypoxia, particularly at night when no photosynthetic production of oxygen occurs.

Hypoxia has been well studied in crustaceans and a good amount of information is available on the processes involved in ion regulation in relation to oxygen saturation. The gills are the most important site for exchange of monovalent ions. The effects of decreased oxygen concentrations on ion regulation can be attributed to the HCO_3^-–Cl^- and Na^+–H^+ ion exchange pathways. The two ions Na^+ and Cl^- are regulated through transport mechanisms that are linked to the transport of H^+ and HCO_3^- ions (Shaw, 1960a, b; Mantel & Farmer, 1983; Truchot, 1983; McMahon, 2002). Evidence, largely gathered from research on freshwater acclimated species (hyper regulators), support the presence of two independent exchange processes: sodium-proton and chloride-bicarbonate. This link between the regulation of the two major haemolymph ions and acid–base concentrations has been established by the fact that crustaceans will show disruption of the ion transport process in response to acid–base disturbance (Cameron, 1978; Wheatly, 1989; Jensen & Malte, 1990). Furthermore, in euryhaline species, changes in ambient salinity, which alter ion exchanges, will result in changes in the acid–base status of the haemolymph (Weiland & Mangum, 1975; Truchot, 1981; Whiteley et al., 2001). In hypoxia, and indeed hyperoxia, the ventilation rate will change and an alteration in the acid–base balance will ensue due to variations in the excretion of CO_2 (Burnett & Johansen, 1981; Hagerman & Uglow, 1982; Wheatly, 1989). Studies investigating the effects of hypoxia in marine or euryhaline species have noticed a decrease in chloride haemolymph concentrations, particularly when the animals were kept in a dilute medium (Hagerman & Uglow, 1982; Johnson & Uglow, 1987; Hagerman & Szaniawska, 1991). Individuals of the three crayfish species kept in this experiment did not exhibit a decrease in chloride concentrations when exposed to hypoxia. Indeed, *A. italicus* individuals kept at 30% oxygen saturation had an increase in haemolymph chloride concentrations. A few authors have found occurrences where exposure to pollutants increased ion regulation in estuarine isopods (Jones, 1975; Oksama & Kristoffersson, 1980). Lignot et al. (2000) suggest that an initial activation of ionic uptake could occur following exposure to pollutants. This hypothesis was put forward by Lignot et al. (2000) to account for the lack of effects in some research or osmotic capacity increases in others (Oksama & Kristoffersson, 1980; Boitel & Truchot, 1989; Ahern & Morris, 1998). It is possible that exposure to small amounts of contaminants results in an increase in ion regulation in crayfish, while higher concentrations of pollutants can produce a decrease in ion uptake, as was observed by Oksama & Kristoffersson (1980). It is also important to note that many authors discussed the effects of a more acute hypoxia on crustaceans. In this study, the levels of hypoxia were intended to reflect what could be encountered in a river with slight or important nutrient and organic enrichment. Wheatly & Taylor (1981) found that below an oxygen partial pressure of 50 mmHg (6.65 KPa), *A. pallipes* reduces its ventilation rate in the gills, most likely owing to the energy cost associated with the beating of the scaphognathites. These authors also found that this species will migrate into air at 42 mmHg or 5.59 KPa (Taylor & Wheatly, 1980). The impact of acute hypoxia or anoxia on osmoregulation in crayfish has yet to be investigated.

Sodium concentrations were found to decrease with hypoxia in *A. pallipes* and *A. torrentium* individuals. Similar results had been obtained in an experiment on *A. pallipes* crayfish from Ireland (Demers, 2003). *A. italicus* crayfish did not exhibit a significant difference in sodium levels in hypoxia. This species might be more tolerant to low oxygen partial pressure. Hildebrandt & Zerbst (1992) found that sodium concentrations in the blood of medicinal leeches decreased significantly after 96 h in hypoxia. However, sodium levels in a brackish water isopod were found not to be affected by hypoxia and Na^+ concentrations decreased only in anoxia (Hagerman & Szaniawska, 1991). The decreased sodium concentrations could also be explained by the energetic cost of the ion pump. Indeed, when confronted to a low oxygen supply, actively pumping ions might be too costly to occur efficiently. Hagerman & Uglow (1981) suggested that the observed loss of haemolymph chloride in *Palaemon adspersus* in hypoxia was caused by the reallocation of energy to other functions. However, further research on *Crangon crangon* showed that this hypothesis was probably too simplistic and that changes in the acid–base balance provided a more likely explanation (Hagerman & Uglow, 1982).

The environmental conditions to which crayfish were exposed did not significantly affect haemolymph calcium concentrations, but the calcium concentrations measured in the haemolymph samples demonstrated great variability between treatments. Changes in haemolymph calcium concentration have been observed in crustaceans exposed to hypoxia (Hagerman & Uglow, 1982; Hagerman & Szaniawska, 1991). A potential mechanism to buffer the acid–base variations which occur in hypoxia is the dissolution of the calcium carbonate in the exoskeleton (Truchot, 1979; Henry & Wheatly, 1992). This adaptation would be valuable as an increase in blood pH and Ca will increase the oxygen affinity of haemocyanin (Mangum, 1980).

A possible bias was introduced to this experiment owing to the fact that *A. italicus* and *A. torrentium* individuals had to travel in air for a much longer period than *A. pallipes* individuals. Particular caution should be taken when considering the *A. torrentium* results, since half of the individuals died during transportation (technically leaving the strongest animals). Two *A. pallipes* kept at 30% oxygen saturation moulted. Wheatly & Ignaszewski (1990) reported that Na^{+} and Cl^{-} influxes were stable throughout the intermoult and premoult phases. However, immediately after ecdysis, these authors demonstrate that there is a net influx of these ions that persists for only two days, Na^{+} and Cl^{-} balance being re-established 3 days postmoult. Therefore, the moult of these two individuals is not believed to have influenced the results as samples were collected 4 days after ecdysis.

A. pallipes usually inhabits fairly cool waters with little temperature variations, although some areas can experience high water temperature in the summer (for example France in 2003). *A. italicus* is found in a warmer climate, being found in Italy and Spain, but the remaining populations usually inhabit headwaters of spring-fed rivers, often at high latitudes. Nevertheless, there is a potential for *A. italicus* to be exposed to higher water temperatures than *A. pallipes*. *A. torrentium* would not experience high temperatures because of its tendency to be found in upland rivers, in the alpine region. Warm water contains less oxygen than cool water, thus crayfish found in the warmer climates might be subjected to hypoxia more often than crayfish living in cool water. This might explain the different responses to hypoxia of the different species.

Nevertheless, crayfish did not seem to be drastically affected by an oxygen saturation of only 30% as a few individuals moulted and none showed external signs of stress such as lack of reaction when touched, decreased activity or sluggishness. Crayfish of this genus seem fairly well adapted to cope with decreased oxygen content for extended periods of time, in this case, 12 days. *A. pallipes* and *A. italicus* have been found in ponds (e.g. Rallo & Garcia-Arberas, 2000) or even in burrows (Peay & Hirst, 2003), which are habitats that can experience low oxygen concentrations. Grandjean et al. (1996) found a well established population of *A. pallipes* in small ponds where dissolved oxygen was measured between 0.8 and 5 mg l^{-1}. Crayfish may thus often encounter moderate hypoxia and should be adapted to deal with low oxygen. Although all three crayfish species experienced a disruption in ionic regulation in response to hypoxia, all individuals survived an extended period of time at a low oxygen concentration. However, these experiments were carried at a constant temperature and it is quite probable that an elevated temperature in combination with low oxygen will have a greater impact on crayfish (Payette & McGaw, 2003; Mugnier & Soyez, 2005).

There is a great need for methods to assess of anthropogenic impacts on aquatic ecosystems. In many European countries, crayfish are assumed to prefer clean, well-oxygenated water. This experiment has shown that crayfish of the genus *Austropotamobius* are quite tolerant to a decrease in ambient oxygen partial pressure and thus to one effect of eutrophication or organic pollution. Their potential use as a bioindicator of water quality is therefore limited. This genus is part of the cultural heritage in several countries in mainland Europe. Because of this, and because of their keystone ecological role, crayfish of the genus *Austropotamobius*, would be better seen as a 'heritage' rather than 'bioindicator' species (Füreder & Reynolds, 2003).

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